

COMPARISON OF THE IDE AND HARRIS (VDRL) SLIDE FLOCCULATION TESTS IN NIGERIA*

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For over 10 years the Ide test has been in use in Nigeria as a simple slide flocculation test for syphilis and yaws in hospitals and clinics, and in the field, where the Kahn and Wassermann tests are impracticable. It was thought desirable that the results obtained by the Ide test should be compared with those obtained by the newly introduced Harris (VDRL) slide flocculation test, and an estimate made of the relative values of the two tests for use in the field.

The sera of 8,077 Nigerian subjects were therefore tested in parallel by the Ide, Harris, and Kahn tests. The Kahn test was used as the basis on which the comparison should be made for two reasons :

(a) It is the test which has been so far the most fully investigated as regards the comparison of clinical with serological findings.

(b) It is the standard routine test in use in this laboratory.

Material

Most of the 8,077 sera were received from the hospitals and clinics of the Lagos area. The sera of yaws cases came from a Yaws Field Survey Unit, and the sera of lepers from a Leper Settlement, both in the Eastern Region of Nigeria. The sources of the sera were as follows :

Source	No. of Sera
Antenatal Clinic (abnormal 451, normal 3,228)	3,679
Routine Medical Examination	420
Blood Donors	108
V.D. and Skin Clinic	395
Eye Clinic	595
E.N.T. Clinic	220
Yaws Cases	204
Leprosy Cases	143
Other Sera	2,313
Total	8,077

(1) Ide Test

When Sobei Ide and Tamao Ide (1936) introduced their colour test for syphilis in Tokyo their

antigen was an alcoholic extract of beef heart sensitized with cholesterol and containing gum benzoin, crystal violet, and azure II. They recommended the use of whole blood diluted with 3.5 per cent. saline and low-power magnification for the reading of the result. In the Nyasaland Protectorate Annual Report for 1938, a modified Ide test was described in which it was claimed that the antigen had been made more sensitive by the replacement of beef heart by sheep heart.

Smith, Elmes, and Smith (1945), in introducing the test to Nigeria, modified it as follows :

- (i) Serum was used instead of whole blood.
- (ii) Inactivation of the serum was suggested but not deemed a necessity.
- (iii) Readings were taken with the naked eye, with or without the use of a slit lamp.

We have used the Smith, Elmes, and Smith method with the two following modifications :

- (i) The serum was always inactivated.
- (ii) The 2.5 per cent. sodium chloride solution used to suspend the antigen was buffered to pH 6.1.

By experiment it was discovered that the results of the tests were unreliable except when the pH of the saline solution lay between 5.9 and 6.3, the optimum being pH 6.1.

Antigen.—This is prepared as described in detail by Smith, Elmes, and Smith (1945), and must be stored in a cool, dark place. The antigen-suspension is prepared by pipetting 1.5 ml. of a 2.5 per cent. solution of sodium chloride in distilled water (buffered to pH 6.1) and 0.5 ml. antigen into separate vials. The saline solution is poured on to the antigen, and, without pausing, the mixture is poured back and forth from vial to vial twelve times, without allowing the vials to drain during the process. The thumb is then placed over the mouth of the mixing vial and the vial is briskly shaken for a few seconds. This antigen-suspension is ready for immediate use, and *must be used within 10 min.* The suspension is first tested against known positive and negative sera, and it is discarded unless clear results are obtained in the control tests.

Serum.—Whole clotted blood is centrifuged and the clear serum removed by a pipette. The serum is heated

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at 56° C. for 30 min. The test should be performed *within 4 hrs of inactivation of the serum*; serum to be tested more than 4 hrs after inactivation must be reheated at 56° C. for a further 10 min. Only clear serum should be used; a serum containing particulate matter should be re-centrifuged.

Test.—The optimal proportion of serum to antigen-suspension is 1 : 2. Mixing on a 3 in. by 1 in. microscope slide is performed with either glass pipettes with rubber teats (preferably standardized by the Starrett Gauge to No. 56) or with platinum wire loops (6 mm.). Separate pipettes (or loops) are used for serum and suspension. The serum pipette is rinsed thoroughly in physiological saline after adding each serum. (If a loop is used, it should be flamed and cooled after each serum.) Two tests may conveniently be done on each slide.

- (i) Place 1 drop (or loopful) serum on slide.
- (ii) Add 2 drops (or loopfuls) antigen-suspension.
- (iii) Rotate slide for 3 min. on a flat surface, through a circle of 2 in. diameter, 120 times per min.
- (iv) Read immediately, holding slide over a dark background and viewing by reflected light with the naked eye (a slit lamp is a distinct advantage).

Interpretation

Reading	Report
No flocculation	Negative (—)
Small blue floccules ..	Positive (+)
Large blue floccules ..	Strongly Positive (++)

(2) Harris (VDRL) Test

We used the VDRL test as described by Harris and others (1946), with slight modifications.

Antigen.—VDRL cardiolipin antigen (Burroughs Wellcome and Co.) was used. It must be stored in a cool, dark place. The antigen-suspension is prepared by pipetting 0.4 ml. of a 1 per cent. solution of sodium chloride in distilled water (buffered to pH 6) to the bottom of a 30-ml. round, glass-stoppered bottle and

adding 0.5 ml. antigen from a 1-ml. pipette (graduated to the tip), the bottle being continuously rotated on a flat surface. The antigen is added drop by drop (taking approximately 6 sec.), the last drop of antigen being blown from the pipette so that the pipette does not touch the surface of the saline solution. The bottle is rotated for a further 10 sec. and 4.1 ml. physiological saline added. The glass stopper having been replaced, the bottle is shaken vigorously for 10 sec. The antigen-suspension is then ready for immediate use and *must be used within 24 hrs*; it should be stored in a cool, dark place. The suspension is first tested against known positive and negative sera, and it is discarded unless clear results are obtained in the control tests.

Serum.—As for the Ide test (*vide supra*).

Test.—The technique is the same as that of the Ide test, except that the optimal proportion of serum to antigen-suspension is 2 : 1.

Interpretation.—The reading and reporting of the result of the test is the same as in the Ide test (except that the floccules are white).

(3) Kahn Test

The Standard (three-tube) Kahn test was employed, "Wellcome" brand Kahn antigen being used throughout.

Results

Of the total 8,077 sera, 1,134 were Kahn-positive. Of these latter, 1,107 (97.6 per cent.) gave a positive result with the Ide test, and 1,124 (99.1 per cent.) gave a positive result with the Harris test. Of the 6,213 sera which were Kahn-negative, 6,071 (97.7 per cent.) were negative to the Ide test, and 6,040 (97.2 per cent.) to the Harris test.

Seven hundred and thirty sera gave a Kahn-doubtful result; of these 505 (69.2 per cent.) gave a positive result to the Ide test, and 565 (77.4 per cent.) a positive result to the Harris test (Table I).

TABLE I
COMPARISON OF KAHN, IDE, AND HARRIS TESTS ON 8,077 SERA

Kahn Test			Ide Test			Harris Test		
Results	Number of Sera	Percentage of total Sera	Results	Number of Sera	Percentage in relation to Kahn Results	Results	Number of Sera	Percentage in relation to Kahn Results
Positive (++++)	289	14.0	Positive	289	97.6 agree	Positive	289	99.1 agree
Positive (+++)	434		Negative	0		Negative	0	
Positive (++)	411		Positive	431		Positive	434	
Doubtful (+ and ±)	730	9.0	Negative	3	69.2 positive	Negative	0	77.4 positive
Negative (—)	6,213	77.0	Positive	387		Positive	401	
			Negative	24	97.7 agree	Negative	10	97.2 agree
			Positive	505		Positive	565	
			Negative	225	97.7 agree	Negative	165	97.2 agree
			Positive	142		Positive	173	
			Negative	6,071		Negative	6,040	
Total	8,077		Total	8,077		Total	8,077	

In Table II the results by the Ide and Harris tests are directly compared, and the χ^2 test is applied. It will be seen that the difference favours the Harris test, but is not highly significant.

TABLE II
COMPARISON OF IDE AND HARRIS TESTS ON 8,077 SERA

Test	Negative	Positive	Total
Ide	6,323	1,754	8,077
Harris ..	6,215	1,862	8,077
Total ..	12,538	3,616	16,154

$$\begin{aligned}\chi^2 &= 4.1558 \\ \text{d.f.} &= 1 \\ 0.05 > P > 0.02\end{aligned}$$

Discussion

These results show that there is very little to choose between the Ide and Harris tests, the balance being slightly in favour of the Harris test. Both tests compare very favourably with the Standard Kahn test.

In the course of the investigation, which occupied nearly a year, we decided that in both the Ide and Harris tests it was possible to make a differentiation in the positive results: many were "strongly positive"; the floccules developed rapidly and became large and very distinct. We are not aware of the significance of these results; the clinical notes were not sufficient in number or detail to allow of a classification on clinical findings. The differentiation, however, was marked enough to warrant a scrutiny of the positive results when divided into "Positive" and "Strongly Positive".

When this differentiation is taken into consideration there is a highly significant difference between the Ide and the Harris tests in favour of the latter (Table III).

TABLE III
COMPARISON OF IDE AND HARRIS TESTS ON 8,077 SERA, DISTINGUISHING "POSITIVE" AND "STRONGLY POSITIVE"

Test	Negative	Positive	Strongly Positive	Total
Ide	6,323	1,056	698	8,077
Harris	6,215	928	934	8,077
Total	12,538	1,984	1,632	16,154

$$\begin{aligned}\chi^2 &= 43.3156 \\ \text{d.f.} &= 2 \\ P &< 0.001\end{aligned}$$

Although, as has been mentioned, the clinical notes attached to the specimens of serum were on the whole insufficient, and the "follow-up" of cases was largely unsuccessful, it was thought worth

while to make the attempt to divide the sera examined into two groups:

- (a) Sera from patients showing some evidence or suspicion of syphilis and/or yaws, present or past.
- (b) Sera from patients showing no such evidence.

Vaughan (1947) states:

The evaluation of serological tests based on a comparison of results obtained by the examination of a series of unselected sera is rendered difficult by the absence of any practicable objective standards... An attempt, however, was made to classify on clinical grounds all the sera examined as either syphilitic or non-syphilitic. The results, being thus based on data open to subjective error, have no absolute value and cannot legitimately (except as an indication of broad trends) be compared with those of other workers.

It is emphasized that our division of the sera into these two groups is based on scanty clinical notes ("data open to subjective error") and was made only in the hope of finding an indication of broad trends.

TABLE IV
COMPARISON OF IDE AND HARRIS TESTS ON 3,468 SERA FROM SUBJECTS SHOWING SOME EVIDENCE OR SUSPICION OF SYPHILIS OR YAWS

Test	Negative	Positive	Total
Ide	2,434	1,034	3,468
Harris ..	2,345	1,123	3,468
Total ..	4,779	2,157	6,936

$$\begin{aligned}\chi^2 &= 5.3296 \\ \text{d.f.} &= 1 \\ P &= 0.02\end{aligned}$$

Table IV shows the comparison of results given by the Ide and Harris tests on 3,468 sera, in which the clinical notes mentioned, or suggested, a history and/or signs and symptoms of syphilis and/or yaws. The Harris test in this group was significantly more sensitive than the Ide test. When the positive results in this group were divided into "Positive" and "Strongly Positive", the greater sensitivity of the Harris test appears to be enhanced (Table V).

TABLE V
COMPARISON OF IDE AND HARRIS TESTS ON 3,468 SERA FROM SUBJECTS SHOWING SOME EVIDENCE OR SUSPICION OF SYPHILIS OR YAWS, DISTINGUISHING BETWEEN "POSITIVE" AND "STRONGLY POSITIVE"

Test	Negative	Positive	Strongly Positive	Total
Ide	2,434	608	426	3,468
Harris	2,345	539	584	3,468
Total	4,779	1,147	1,010	6,936

$$\begin{aligned}\chi^2 &= 30.5250 \\ \text{d.f.} &= 2 \\ P &< 0.001\end{aligned}$$

When 3,993 sera from subjects showing no evidence or suspicion of syphilis or yaws were

examined there was no significant difference between the two tests (Table VI), but when the

TABLE VI

COMPARISON OF IDE AND HARRIS TESTS ON 3,993 SERA FROM SUBJECTS SHOWING NO EVIDENCE OR SUSPICION OF SYPHILIS OR YAWS

Test	Negative	Positive	Total
Ide	3,404	589	3,993
Harris ..	3,392	601	3,993
Total ..	6,796	1,190	7,986

$$\chi^2 = 0.1422$$

$$\text{d.f.} = 1$$

$$0.8 > P > 0.7$$

positive sera in this group were divided into "Positive" and "Strongly Positive" there was a highly significant difference between the tests, the Harris test being the more specific (Table VII).

TABLE VII

COMPARISON OF IDE AND HARRIS TESTS ON 3,993 SERA FROM SUBJECTS SHOWING NO EVIDENCE OR SUSPICION OF SYPHILIS OR YAWS, DISTINGUISHING "POSITIVE" AND "STRONGLY POSITIVE"

Test	Negative	Positive	Strongly Positive	Total
Ide	3,404	376	213	3,993
Harris	3,392	327	274	3,993
Total	6,796	703	487	7,986

$$\chi^2 = 11.0772$$

$$\text{d.f.} = 2$$

$$0.01 > P > 0.001$$

One reason for differentiating between "Positive" and "Strongly Positive" results in the Ide and Harris tests was to seek for light upon the problem of "the doubtful Kahn test"—a problem affecting both serologists and clinicians, especially the latter. Our figures, though interesting, are of no real value, again because, without adequate clinical correlation, positive Ide and Harris results by themselves (without reference to simultaneous Kahn results) are of unknown reliability.

Conclusions

The following conclusions have been reached after considerable experience of the two tests in a well-equipped and well-staffed serology laboratory; they await confirmation after more extensive use in parallel in small field laboratories.

(1) In comparison with the Kahn test, the Harris test is slightly more specific than the Ide test.

(2) In the examination of sera from subjects showing evidence of syphilis or yaws, the Harris test is more sensitive than the Ide test.

(3) When grades of positivity are introduced, the Harris test is significantly more specific and more sensitive than the Ide test.

(4) The Harris test, when used in screening, would be more sensitive than the Ide test.

(5) There is no difference in the skill required for the performance of the two tests.

(6) The fact that the antigen-suspension in the Harris test can be used up to 24 hrs after mixing (as compared with 10 min. in the Ide test) is an advantage in field work.

(7) The Ide antigen is simple and inexpensive to manufacture, as compared with the complex and costly Harris antigen.

(8) There appears to be no difference in the keeping properties of the two antigens.

Summary

(1) The essential points in the techniques of the Ide test and Harris (VDRL) test are described.

(2) The results are given of the examination of 8,077 sera from Nigerian subjects by the Ide and Harris tests in parallel with the Standard Kahn test.

(3) The Ide test showed a 97 per cent. agreement with the Kahn test when testing Kahn-positive and Kahn-negative sera. Of Kahn-doubtful sera, 69 per cent. gave a positive result with the Ide test. The Harris test showed a 99 per cent. agreement with the Kahn test when testing Kahn-positive sera, and a 97 per cent. agreement when testing Kahn-negative sera. Of Kahn-doubtful sera, 77 per cent. gave a positive result with the Harris test.

(4) The results of the two tests in examining "syphilitic" and "non-syphilitic" sera, and the question of degrees of positivity in the two tests are discussed.

(5) The conclusions drawn from a comparison of the Ide and Harris tests are enumerated.

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